PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 501/12, C12P 35/04 — A1

(11) International Publication Number: **WO 99/31109**

(43) International Publication Date: 24 June 1999 (24.06.99)

(21) International Application Number: PCT/NL98/00714

(22) International Filing Date: 14 December 1998 (14.12.98)

(30) Priority Data:
1007828 18 December 1997 (18.12.97) NL

(71) Applicant *(for all designated States except US)*: DSM N.V. [NL/NL]; Het Overloon 1, NL–6411 TE Heerlen (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KEMPERMAN, Gerjan [NL/NL]; Hoogmeer 19–50, NL–6605 CM Wijchen (NL). DE GELDER, René [NL/NL]; Jonathan 59, NL–6662 JJ Elst (NL). RAEMAKERS–FRANKEN, Petronella, Catharina [NL/NL]; Europalaan Zuid 124, NL–6021 KJ Budel (NL).

(74) Agent: JACOBS, Monique, Sophie, Nicole; Octrooibureau DSM, P.O. Box 9, NL–6160 MA Geleen (NL).

(81) Designated States: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
In English translation (filed in Dutch).

(54) Title: COMPLEXES OF ß–LACTAM ANTIBIOTICS AND 1–NAPHTHOL

(57) Abstract

The invention relates to complexes of cephradine and cefaclor and 1–naphthol. It has been found that 1–naphthol shows better complexing behaviour then for example 2–naphthol. The invention also relates to a process for the preparation of such complexes, the corresponding ß–lactam antibiotic being prepared through acylation of the corresponding ß–lactam nucleus with a suitable acylating agent and 1–naphthol being present in the reaction mixture during at least part of the acylation reaction. The acylation is preferably carried out in the presence of an enzyme. The ß–lactam antibiotic can subsequently be released from the complex in a known manner.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

COMPLEXES OF β-LACTAM ANTIBIOTICS AND 1-NAPHTHOL

The invention relates to complexes of β-lactam antibiotics chosen from the group comprising cephradine and cefaclor, and 1-naphthol.

Complexes of β-lactam antibiotics and hydroxynaphthalenes are known in general terms from WO-A-93/12250, which explicitly describes the complexes of cephalexine and cephadroxyl, and 2-naphthol.

The applicant has now found that the formation of a complex of cephradine and cefaclor, which β-lactam antibiotics proved to show comparable complexing behaviour, proceeds faster and more completely with 1-naphthol than with 2-naphthol, whereas the opposite holds for other β-lactam antibiotics.

The complexes according to the invention are in particular useful intermediates, for example in the enzymatic preparation of cephradine and cefaclor, in the recovery of the β-lactam antibiotics from reaction mixtures obtained after a chemical or enzymatic acylation reaction and the purification of β-lactam antibiotics. Cephradine is a β-lactam antibiotic that can be obtained through acylation of 7-aminodesacetoxycephalosporanic acid (7-ADCA) with D-dihydrophenylglycine or a derivative thereof, for example an amide or an alkyl ester, preferably a lower (1-4 C) alkyl ester; cefaclor is a β-lactam antibiotic that can be obtained through acylation of 7-amino-3-chloro-ceph-3-em-4-carboxylic acid with D-phenylglycine

or a derivative thereof, preferably a lower (1-4 C) alkyl ester, or an amide.

The complexes according to the invention can be prepared in a simple manner by bringing the β-lactam antibiotic into contact with 1-naphthol. The molar ratio of the 1-naphthol and the β-lactam antibiotic is preferably greater than 0.5 and is in particular between 0.5 and 2. The concentration of the β-lactam antibiotic is preferably chosen to be as high as possible, preferably greater than 0.01 wt.% β-lactam antibiotic in the reaction mixture. The temperature applied is not particularly critical and is for example between -10 and 100°C, preferably between -5 and 50°C. The pH at which the complexes are formed is not particularly critical either; the residual concentration of the β-lactam antibiotic in solution to be obtained after complexing with 1-naphthol proves to be virtually independent of the mixture's pH in a wide range of pH values, for example between 1 and 10, in particular 2 and 9, more in particular 3 and 8. That complex formation can consequently be incorporated in a simple manner at various points in a process for the preparation of β-lactam antibiotics, for example during an enzymatic acylation reaction, in the hydrolysis of protected β-lactam antibiotics after a chemical acylation reaction in which use is made of protecting groups, in the purification of antibiotics or in the isolation of β-lactam antibiotics from a reaction mixture obtained after the acylation reaction or from the mother liquor. Preferably a pH value of between 2 and 9, in particular between 4 and 7, is chosen. The β-lactam antibiotic can be recovered from the complex in

a manner that is generally known to those skilled in the art.

A particularly suitable application of the complexes according to the invention is in the enzymatic acylation of a β-lactam nucleus with an acylating agent, 1-naphthol being present in the reaction mixture during at least part of the acylation reaction. With a kinetically controlled coupling, hydrolysis of the acylating agent and the β-lactam antibiotic usually occurs during an enzymatic acylation reaction. Owing to the presence of 1-naphthol in the reaction mixture, and consequently the formation of the complexes according to the invention, a higher synthesis/hydrolysis ratio (S/H), the molar ratio of the synthesis product (β-lactam antibiotic) and the hydrolysis product, is obtained and less decomposition of the β-lactam antibiotic occurs as a result of the complexing. In addition, a higher reaction rate was realised, as a result of which the decomposition of the β-lactam antibiotic and the β-lactam nucleus is restricted.

The concentration at which the enzymatic acylation reaction is carried out is not particularly critical. The concentration of the β-lactam nucleus and of the acylating agent at the beginning of the acylation reaction is for example between 100 and 2,000 mM, preferably between 400 and 1,000 mM. Preferably the β-lactam nucleus and/or the acylating agent are during at least part of the acylation reaction present in the reaction mixture in a supersaturated form. This can for example be realised by subjecting a mixture in which the β-lactam nucleus and/or the acylating agent are

present in a concentrated form to an increase or reduction in pH or to a reduction in temperature.

As the enzyme in the acylation reaction any enzyme can in principle be used that is suitable for use as a catalyst in the coupling reaction. Such enzymes are for example the enzymes known under the general name of penicillin amidase or penicillin acylase. Such enzymes are for example described in J.G. Shewale et al., Process Biochemistry, August 1989, pp. 146-154, and in J.G. Shewale et al., Process Biochemistry International, June 1990, pp. 97-103. Examples of suitable enzymes are enzymes derived from Acetobacter, in particular Acetobacter pasteurianum, Aeromonas, Alcaligenes, in particular Alcaligenes faecalis, Aphanocladium, Bacillus sp., in particular Bacillus megaterium, Cephalosporium, Escherichia, in particular Escherichia coli, Flavobacterium, Fusarium, in particular Fusarium oxysporum and Fusarium solani, Kluyvera, Mycoplana, Protaminobacter, Proteus, in particular Proteus rettgeri, Pseudomonas and Xanthomonas, in particular Xanthomonas citrii.

Preferably use is made of an immobilised enzyme, because the enzyme can then be separated and reused in a simple manner.

Of the commercially available immobilised enzymes, the Escherichia coli enzyme of Boehringer Mannheim GmbH that is commercially available under the name of Enzygel®, the immobilised Penicillin-G acylase of Recordati and the immobilised Penicillin-G acylase of Pharma Biotechnology Hannover for example have proved to be very suitable. Enzymes can also be used as a crystalline substance (CLECs™).

The temperature at which the enzymatic acylation reaction is carried out is not particularly critical and is, on account of the enzyme's stability, usually lower than 40°C, preferably between -5 and 35°C. The pH at which the enzymatic acylation reaction is carried out is usually between 5.5 and 9.5, preferably between 6.0 and 9.0.

Preferably the reaction is almost completely stopped as soon as almost the maximum degree of conversion has been reached. A suitable mode of stopping the reaction is lowering the pH, preferably to a value of between 4.0 and 6.3, in particular between 4.5 and 5.7. Another suitable mode is lowering the temperature of the reaction mixture as soon as the maximum degree of conversion has been reached. A combination of the two modes is also possible.

After the reaction has been virtually stopped when the maximum degree of conversion has been reached, the reaction mixture is usually present in the form of a suspension containing several solid substances, for example the antibiotic and D-phenylglycine, while immobilised enzyme may also be present. The immobilised enzyme is preferably recovered, in view of process economics. This can for example be carried out in a suitable manner by filtering the reaction mixture through a sieve, with stirring, the stirrer's direction of rotation preferably being chosen so that the suspension is pumped upwards at the centre of the stirrer. Valuable components, for example the antibiotic and PG, can subsequently be recovered, for example with the aid of a change in pH.

A reduction in pH can in the context of the invention for example be effected by adding an acid. Suitable acids are for example mineral acids, in particular sulphuric acid, hydrochloric acid or nitric acid, and carboxylic acids, for example acetic acid, oxalic acid or citric acid. An increase in pH can for example be effected by adding a base. Suitable bases are for example inorganic bases, in particular ammonia, potassium hydroxide or sodium hydroxide, and organic bases, for example triethylamine and D-phenylglycine amide. Preferably ammonia is used.

The enzymatic acylation reaction and the indicated measures, for example the preparation of the supersaturated mixtures, can be carried out in water. The reaction mixture may optionally also contain an organic solvent or a mixture of organic solvents, preferably less than 30 vol.%. Examples of organic solvents that can be used are alcohols with 1-7 C atoms, for example a monoalcohol, in particular methanol or ethanol; a diol, in particular ethylene glycol, or a triol, in particular glycerol.

The molar ratio of the acylating agent and the β-lactam nucleus, i.e. the total amount of acylating agent supplied divided by the total amount of β-lactam nucleus supplied expressed in moles, is smaller than 2.5. Preferably the molar ratio is between 0.5 and 2.0, in particular between 0.7 and 1.8.

The enzymatic acylation reaction is preferably carried out as a batch process. It is optionally also possible to carry out the reaction continuously.

The invention will be further elucidated with reference to the examples without however being limited thereby.

Examples

7-ACCA:	7-amino-3-chloro-ceph-3-em-4-carboxylic acid
7-ADCA:	7-aminodesacetoxycephalosporanic acid
6-APA:	6-aminopenicillanic acid
CCl:	cefaclor
CEX:	cephalexine
PG:	D-phenylglycine
PGA:	D-phenylglycine amide
HPG:	D-p-hydroxyphenylglycine
HPGM:	D-p-hydroxyphenylglycine methyl ester

Assemblase™ is an immobilised Escherichia coli penicillin acylase from E. coli ATCC 11105, as described in WO-A-97/04086. The immobilisation was carried out as described in EP-A-222462, using gelatine and chitosan as the gelling agents and glutaraldehyde as a crosslinker.

The ultimate activity of the Escherichia coli penicillin acylase is determined by the amount of enzyme added to the activated spheres and was 3 ASU/g of dry weight, 1 ASU (Amoxicillin Synthesis Unit) being defined as the amount of enzyme that generates 1 g of Amoxicillin.$3H_2O$ per hour from 6-APA and HPGM (at 20°C; 6.5% 6-APA and 6.5% HPGM).

Example I

Complexing of cephradine using 1-naphthol or 2-naphthol (comparative experiment) as the complexing agent.

A basic solution was added, drop by drop, to a(n aqueous) solution of cephradine having a concentration of 1.0 m.% until a pH of 6.3 was obtained. Next, an equimolar amount of 1-naphthol or 2-naphthol was added at room temperature.

Samples were taken at different moments during a stirring incubation. After these samples had been filtered through a 0.45 μ filter, the concentration of the cephalosporine in question present in the filtrate was determined with the aid of HPLC. The results are presented in Table 1.1.

Table 1.1

Complexing of cephradine using 1-naphthol or 2-naphthol as the complexing agent.

reaction time (hours)	[cephradine] in filtrate (m.%)			
	0	0.5	1.5	24
1-naphthol	1.0	0.65	0.05	0.03
2-naphthol*	1.0	0.80	0.65	0.65

* comparative experiment

Example II

Example I was repeated for cefaclor instead of cephradine; now at a pH of 7.0.

The results are presented in Table 1.2.

Table 1.2

Complexing of cefaclor using 1-naphthol or 2-naphthol as a complexing agent (pH 7.0, room temperature).

reaction time (hours)	[cefaclor] in filtrate (m.%)			
	0	0.5	1.5	24
1-naphthol	1.05	0.72	0.38	0.13
2-naphthol*	1.02	0.47	0.43	0.22

* comparative experiment

Comparative Experiment A

Example I was repeated for cefadroxil instead of cephradine. The results are presented in Table 1.3.

Table 1.3

Complexing of cefadroxyl using 1-naphthol or 2-naphthol as the complexing agent.

reaction time (hours)	[cefadroxyl] in filtrate (m.%)			
	0	0.5	1.5	24
1-naphthol	1.0	0.98	0.80	0.73
2-naphthol	1.0	0.49	0.47	0.42

Example III

Complexing of cephradine using 1-naphthol as the complexing agent at various pH values.

A(n aqueous) cephradine solution having a(n initial) concentration of 1.8 percent by mass was divided between 3 reaction vessels. With the aid of a diluted sulphuric acid solution the cephradine solution in one of the reaction vessels was brought to a pH of 4.5. The pH values of the cephradine solutions in the other reaction vessels were brought to 6.3 and 7.0 respectively, with a diluted ammonia solution. Next, an equimolar amount of 1-naphthol was added at room temperature. Samples were taken at various moments during a stirring incubation.

The concentrations of cephradine present in filtrate samples are indicated in Table 2.1.

Table 2.1

Complexing of cephradine using 1-naphthol as a complexing agent at various pH values.

reaction time (hours)	[cephradine] in filtrate (m.%)											
	0			0.5			1.5			24		
pH	4.5	6.3	7.0	4.5	6.3	7.0	4.5	6.3	7.0	4.5	6.3	7.0
1-naphthol	1.8	1.8	1.8	1.64	1.26	1.22	0.84	0.88	1.13	0.79	0.78	1.05

Example IV

To an aqueous solution (temperature 10°C) containing 10 g of PGA (454 mM) and 11.3 g of 7-ACCA (328 mM) was added 3.4 g of 1- or 2-naphthol (160 mM) at T = 10°C.

Before Assemblase™ (24 g) was added, the pH was brought to 7.0 with the aid of 2N H_2SO_4. Samples were taken at various moments and analysed with the aid of HPLC. The degree of conversion (the number of moles of cefaclor formed relative to the number of moles of 7-ACCA with which the reaction began) is shown in Table 3.1.

Table 3.1

Enzymatic synthesis of cefaclor in the presence of 1-naphthol or 2-naphthol (10°C)

	Degree of conversion (%)				
Time (min.)	2	15	29	56	176
1-naphthol	4.8	16.6	34.8	59.1	>90
2-naphthol	0.9	9.7	19.1	40.2	84.6

CLAIMS

1. Complex of a β-lactam antibiotic chosen from the group comprising cephradine and cefaclor and 1-naphthol.
2. Process for the preparation of a complex according to Claim 1, in which the corresponding β-lactam antibiotic is brought into contact with 1-naphthol.
3. Process for the preparation of a complex according to Claim 1, in which the corresponding β-lactam antibiotic is prepared through acylation of the corresponding β-lactam nucleus with a suitable acylating agent and in which 1-naphthol is present in the reaction mixture during at least part of the acylation reaction.
4. Process according to Claim 3, in which the acylation is carried out in the presence of an enzyme.
5. Process according to Claim 3 or Claim 4, in which the complex is isolated and the β-lactam antibiotic is released from the complex.
6. Process for the recovery of a β-lactam antibiotic chosen from the group comprising cephradine and cefaclor, a mixture containing the β-lactam antibiotic being brought into contact with 1-naphthol and the complex formed being recovered.
7. Process according to Claim 6, the β-lactam antibiotic subsequently being released from the complex.

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/NL 98/00714

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D501/12 C12P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 003 896 A (P. FAARUP) 18 January 1977 see claim 3; example 2	1-7
Y	WO 93 12250 A (NOVO NORDISK A/S) 24 June 1993 cited in the application see claims	1-7
A	CHEMICAL ABSTRACTS, vol. 84, no. 21, 24 May 1976 Columbus, Ohio, US; abstract no. 150644, KODAMA T. ET AL.: "Purification of cephalosporins" XP002059554 see abstract & JP 50 130778 A (TOYAMA CHEMICAL CO.,LTD.)	1-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 March 1999	19/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chouly, J

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]onal Application No
PCT/NL 98/00714

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4003896 A	18-01-1977	NONE	
WO 9312250 A	24-06-1993	AU 3345193 A	19-07-1993
		DE 618979 T	18-05-1995
		EP 0618979 A	12-10-1994
		ES 2059285 T	16-11-1994
		GR 94300079 T	30-11-1994
		JP 7502168 T	09-03-1995
		PL 170842 B	31-01-1997
		SK 63894 A	08-03-1995
		US 5470717 A	28-11-1995